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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER

SGAGIAS, MAGDALENE K

ART UNIT	PAPER NUMBER
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1632

NOTIFICATION DATE	DELIVERY MODE
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ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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<p align="center">Advisory Action Before the Filing of an Appeal Brief</p>	<p>Application No. 10/518,749</p>	<p>Applicant(s) NAKAYAMA ET AL.</p>	
	<p>Examiner MAGDALENE K. SGAGIAS</p>	<p>Art Unit 1632</p>	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 02 July 2008 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:

- a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
(a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
(b) ☐ They raise the issue of new matter (see NOTE below);
(c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
(d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
The status of the claim(s) is (or will be) as follows:
Claim(s) allowed: _____.
Claim(s) objected to: _____.
Claim(s) rejected: _____.
Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☒ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because:
See Continuation Sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____.
13. ☐ Other: _____.

/Anne-Marie Falk/
Anne-Marie Falk, Ph.D.
Primary Examiner, Art Unit 1632

Continuation of 11. does NOT place the application in condition for allowance because:

A. Applicants argue the cells described as "embryonic stem cells" in Weiss are primary neural cells, as prepared in Example 3. In other words, the primary neural cells are already directed to ectodermal neural cells by the process of the development of an individual (stem cells prepared from embryo or neural stem cells from embryo. On the other hand, the "embryonic stem cells" which are the subject of the present invention have an ability of differentiating into all of the cells, ectoderm, endoderm, or mesoderm, in addition, the embryonic stem cells are cells established as a cell line from an inner cell mass of blastocysts, so that they are clearly distinguishable from the primary neural cells in Weiss. Therefore, Weiss using the cells directed to ectodermal neural cell, i.e. cells already destined to one direction, is essentially different from the present invention using cells capable of differentiating into all of the cells, ectoderm, endoderm, or mesoderm. Thus, the present invention cannot be anticipated from Weiss.

These arguments are not persuasive because Weiss et al in example 3 teach isolation and propagation of embryonic stem cells from embryonic day 14 (E14) CD albino mice. The claimed invention is not limited to the source of embryonic stem cells and furthermore their source is not limited to the inner cell mass of blastocysts. The specification on page 19 teaches "The embryonic stem cells used in the method for producing a neural cell of the present invention is not particularly limited in the kind of individual to be a resource of the cell).

Applicants argue Weiss describes that the differentiation into DA-neurons occurs in the presence of FGF-2, as described in various parts of Weiss (Abstract; column 1, lines 44-50; column 2, lines 56-67; Example 2: column 12, lines 2-6; Example 8: column 15, lines 1-15; and lines 19-23). Weiss does not contain any description at all on the differentiation into DA-neurons under the conditions of "ACM," "ACM+EGF," "ECM," or "BCM+EGF." On the other hand, in the present invention, the differentiation into neural cells occurs only with astrocyte-conditioned medium (ACM).

These arguments are not persuasive because the invention as claimed requires the isolated neuron to express tyrosine hydroxylase and Weiss et al teach the isolated neuron expresses tyrosine hydroxylase as in the claimed invention. In addition, Weiss teaches the presence of ingredients equivalent to astrocyte conditioned medium as in the claimed invention where the cells are isolated in a suspension of embryonic stem cells in the presence of ingredients equivalent to an astrocyte conditioned medium in the state of adhesion of the neural stem cells to an adhesive culture substratum by plating the cells onto poly-L-ornithine coated glass cover slips, in the complete medium with rat B49 glial cell line-derived conditioned medium in the absence of bFGF, in the presence of FGF2 as in the claimed invention. Therefore, the rejection is maintained.

B. Applicants argue Zhang reports that human embryonic stem (ES) cells cannot be maintained in an undifferentiated state in the absence of FGF-2 (Ref. 4 of Zhang). From this report, it is considered in Zhang that the differentiation occurs by removing FGF-2 and forming embryonic bodies (EB). Therefore, if FGF-2 is added, the differentiation would have been suppressed.

These arguments are not persuasive because Ref 4 of Zhang by Amit is entitled "Clonally derived human embryonic stem cell lines maintain pluripotency and proliferating potential for prolonged periods of culture" but in the instant case the Zhang reference is not used for such type of rejection but for the use of ingredients equivalent to the conditioned medium and the absence of EGF as required in the instant claimed invention.

Applicants argue the neural stem sphere used in the present invention, the suppression of differentiation into the neural stem cells (NSC) does not take place even when FGF-2 is added upon suspension culture in ACM. Thus, the present invention possesses a synergistic effect, contrary to that described in Zhang, which enhances the differentiation. The effect for FGF-2 upon the suspension culture as described above is reversed; therefore, it is clear that the present invention is completely different from that taught in Zhang. The method of Zhang allows differentiation of the cells by an EB method to form neural tube-like structure on day 7 of the adhesion culture. By contrast, in the present invention, a sufficient amount of neural stem cells (NSC) are differentiated day 4 after the suspension, more in the suspension culture, not the adhesion culture. In the present invention, it is reasonable to consider that the reason that the NSC are differentiated only on a surface layer of the neural stem sphere is that a factor in ACM penetrates into the neural stem sphere, thereby enhancing the differentiation into NSC, proving that the ES cells are subjected to direct differentiation. The NSC are reportedly neuroepithelial stem cells and radial glia of the ventricular zone in embryo, or astrocytes of the subventricular zone and subgranular zone in adult (See Attachment 1: Doetseh, F., Nature Neurosci 6, 1127-1134, 2003). The fact that the NSC are differentiated in the order of neuroepithelial stem cells --> radial glia --> astrocytes, strongly suggests that the differentiation into astrocytes occurs in a default state of brain development. Therefore, the fact that almost all of the ES-derived NSC is differentiated into astrocytes by removing FGF-2 agrees with the phenomenon in the development of the brain in a living body.

These arguments are not persuasive because Zhang reference is used in the instant rejection for the claimed invention of claims 1-3 and 10-12 as cited in the office action pages 5-6 and specifically for the limitations of ingredients equivalent to the conditioned medium and the absence of EGF as required in the instant claimed invention (see for example claim 10). The effect of FGF2 upon differentiation or time of differentiation or order of differentiation of stem cells is not a requirement of the claimed invention.

Applicants argue upon the differentiation into the neural cells in the brain, firstly the differentiation into neurons occurs, and subsequently the differentiation into astrocytes and oligodendrocytes occurs in accordance with the progress of the time axis (See Attachment 2: Temple, S., Nature 414, 112-117, 2001). The ES-derived NSC of the present invention are differentiated into astrocytes in a default state as mentioned above, while almost all of the cells are differentiated into neurons by providing an exogenic differentiation stimulation called ACM. The NSC of Zhang is differentiated into three kinds of neural cells, namely neurons, astrocytes, and oligodendrocytes. Taking into consideration the axis of time from the development of the brain, it is understood that the neural stem cells of the present invention are

more undifferentiated than those of Zhang, so that the cells of the present inventions seem to exhibit the nature close to that of the neuroepithelial stem cells. In order that small elongated cells congregated in the center shown in Fig. 1-A of Zhang form a neural tube-like structure shown in Fig. 1-B with the time course, Zhang merely mentions an experimental tool to study human neural tube formation under controlled conditions (Zhang, p. 1131, second column, second paragraph), i.e. ES cell-derived neural precursor cells, recapitulate early steps of nervous system development in that neural tube-like structures are formed, merely stating that the process of development is reproduced. Therefore, Zhang does not directly relate to the differentiation of the ES cells into NSC. Flat cells are migrated in the periphery of the adherent EBs; however, these cells are negative against markers for neurons, astrocytes, oligodendrites, and ES cells. Therefore, under the conditions of Zhang, many of ES cells are differentiated into unidentified cells; therefore, it is obvious that the ES cells cannot be directly differentiated into NSC. Art advantage of the Zhang method is to collect the cells only having a rosette structure utilizing the difference in adhesion from unidentified fiat cells, but never describing that Zhang performs direct differentiation of the ES cells.

Again these arguments are not persuasive because the instant invention does not require a temporal information for neural stem cell differentiation into neurons, astrocytes or oligodendrocytes as described in the reference of Temple. Zhang teaches human ES cells generate all three CNS cell types in vitro and expanded as neurospheres as required in the instant invention in a medium equivalent to the astrocyte conditioned medium. It is not required in the present invention a certain percentage of ES cells differentiating into NSC, or a certain region of the neurosphere (central or peripheral region of the neurosphere) having the NSC, therefore whether the percentage of differentiated cells in Zhang reference is different from that of the instant invention is irrelevant.

Applicants argue the article contribution of Flax describes NSC collected from human fetal telencephalon that is cryopreserved, so that Flax is distinguishable from the teachings of the present invention in the cell species.

These arguments are not persuasive because it would have been obvious for an ordinary of skill in the art to use conditions of Flax for the cryopreservation of NSC as for human fetal telencephalon of Flax. Therefore, the instant rejection is maintained.

C. Rejections under 35 USC § 102/103

Applicants argue Pataky does not cure the deficiencies discussed with regard to Zhang. Pataky reports that neuronal axons of the CNS are regenerated in the spinal cord injuries (Refs. 65 and 66 of Pataky). While Pataky makes references to these publications, the main theme of the article is to evaluate what sort of factors enhance survival in regenerable bulbospinal neurons against injuries caused by axotomy. The effect of enhancing survival of ACM is such that astrocyte-conditioned medium also enhances the survival of bulbospinal neurons, supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro (page 366, second paragraph, last sentence of Pataky), to expect the enhancement of the survival by nonneuronal cells (including astrocytes) in the periphery of the injured spinal neurons. The bulbospinal neurons prepared from E8 Embryo retrograde-labeled with Dil have already ended differentiating into neurons (within the developing chick brain stem, neurogenesis is complete prior to E5; page 367, first paragraph, first sentence of Pataky), so that outgrowth of neurites from bulbospinal neurons is caused by regeneration. Therefore, Pataky which acknowledges that the differentiation into neurons is ended can no way expect the effect of nerve cell differentiation in ACM.

In response to applicant's argument that references 65 and 66 of Pataky the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981). Zhang differs from the claimed invention by not teaching the culturing of stem cell spheres in the presence and then in the absence of bFGF and/or EGF for obtaining glial cell as a cell migrating from the stem cell sphere. Pataky teaches that fibroblast growth factor produced differential effects on survival and neurite outgrowth from identical bulbospinal neurons in vitro. Pataky teaches that astrocytes synthesize a variety of trophic factors and astrocyte conditioned medium also promoted the survival of bulbospinal neurons. Therefore, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the neural stem cell methodology of Zhang by progressive steps of adding bFGF or EGF to obtain neural stem cells and then culture the stem cells in the presence of bFGF or EGF to obtain glial cells with a reasonable expectation of success because Pataky states that astrocytes produce neural nutritional factors such as FGF2. Other teachings of Pataky with regard to ACM also enhances the survival of bulbospianl neurons supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro does not interfere with the use of Pataky reference for making up the deficiency of Zhang for culturing cells of stem cell spheres in the presence and then in the absence of bFGF and/or EGF for obtaining glial cells as a cell migrating from the stem cell sphere. Therefore, the rejection is maintained.